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AN ELECTROPHORETIC COMPARISON OF THE HISPANIOLAN LIZARDS *ANOLIS CYBOTES* AND *A. MARCANOI*

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ABSTRACT. Samples representing four localities — one for both species, two for *A. marcanoi,* and one for *A. cybotes* — were examined. Results for 24 polypeptides are reported, of which 21 were studied in all individuals. With each of 10 proteins individual identification is unequivocal or nearly so. These data confirm the presence of two species in Peravia Province of the Dominican Republic, verify the recognition of the red-dewlapped form as the new species *A. marcanoi,* and indicate that successful hybridization and introgression must be rare, if they occur at all.

Anolis cybotes and the newly described *A. marcanoi* (Williams, 1974) are so similar in morphology that no scale character will consistently separate them. The latter was recognized only because its red dewlap contrasts with the yellow one of the former. For anoles such a difference in dewlap color probably is important for reproductive isolation (Rand and Williams, 1970; Webster and Burns, 1973). In addition, populations of the two have been found side by side, but individuals are not known to mingle freely. This interaction, which is characteristic of closely related anoles, and the difference in dewlap color together provide sufficient evidence for the description of *A. marcanoi.* However, the great similarity of the two species invites additional information on the extent to which they have diverged and perfected reproductive isolation. I report here a study that used starch gel electrophoresis to examine some of their enzymes and nonenzymatic proteins.

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MATERIALS AND METHODS

Seven samples were examined. Of 62 individuals collected in October 1970 by T. A. Jenssen in the vicinity of San José de Ocoa, Peravia Province, Dominican Republic, 42 were red-dewlapped *A. marcanoi* (sample 3a) and 20 were yellow-dewlapped *A. cybotes* (sample 4a). In July 1974 E. E. Williams, R. B. Huey, P. E. Hertz, and R. Holt collected the remaining Peravia Province samples: additional short series of both species from San José de Ocoa (samples 3b and 4b) and *A. marcanoi* from La Gina (sample 1) and from the type locality, 5 km N of La Horma (sample 2). Sample 5 consists of 4 individuals from Debarasse, Departement du Sud, Haiti, a locality a few kilometers to the west of Jérémie, the type locality for *A. cybotes*. The Jenssen collection was shipped alive to Cambridge where the lizards were bled and frozen, but all other series were frozen in the field.

Methods of sample preparation and horizontal starch gel electrophoresis are derived from Selander *et al.* (1971). Protein stains and specific assays are similar to those current in work with vertebrates. Procedural details such as buffer systems best suited for each protein and minor modifications to published assay formulas are available from the author. With the exception of hemoglobin and a plasma protein, all proteins were examined in tissue homogenates. For some proteins, particularly indophenol oxidase, better results were obtained from lizards frozen in Cambridge than from those frozen in Hispaniola.

In many reports on genetic differentiation between vertebrate populations, including an earlier report on *Anolis* species (Webster, Selander, and Yang, 1972), the results are expressed as values of Rogers' coefficient of genetic similarity, S (Rogers, 1972). Unfortunately, in some circumstances the effect of this formula is counterintuitive. When a single locus is considered and no alleles are shared by two populations, the expected similarity is 0. If both populations are polymorphic, however, S is nonzero. The results of this study are presented as Nei's normalized identity of genes, I (Nei, 1972), which is consistently somewhat (2–7%) larger than S calculated for the same data.

For the computation of I, each polypeptide is treated as the product of a single gene.

RESULTS AND DISCUSSION

Among the polypeptides examined in whole animal homogenates, the bands representing 21 could be interpreted with sufficient consistency to be used in estimating relationships. Of these, eight indicate complete or almost complete differentiation of all populations of *A. marcanoi* from those of *A. cybotes* (Table 1). In addition, samples 3a and 4a apparently do not share variants of hemoglobin, plasma protein-1, and indophenol oxidase. For four of these proteins (hemoglobin, plasma protein-1, protein A, and lactate dehydrogenase-1) the difference in electrophoretic mobility is consistent, but so small that an individual expressing both variants could be confused with one producing a single variant. The differences for 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase-1, phosphoglucomutase-1, alcohol dehydrogenase, albumin, and peptidase can be scored unequivocally.

Samples 3b and 4b and the majority of individuals in samples 3a and 4a were collected 2 km S of San José de Ocoa, along the bed and banks of the Rio Ocoa. At this locality the two species are common and in close contact. In such situations of parapatry or sympatry, discrete variation in the electrophoretic mobility of proteins can be more informative than morphological differentiation. Without genetic analysis or biochemical study of protein structure, interpretation of observed differences as allelic variation is generally correct (see Johnson, 1973, for criticism and enumeration of exceptions). Indeed, the inheritance of interspecific differences in some proteins has been observed in natural *Anolis* hybrids (Gorman *et al.*, 1971; Webster, unpublished); and patterns of phenotypic variation in anole populations can be explained by simple molecular and Mendelian models. Differences in phenotypic frequencies thus indicate the presence of reproductive isolation. Detection of isolation does not depend on absolute separation and could be inferred even from significant differences in allelic frequencies at a few loci. For these samples, each of 11 loci indicates an absence of allelic exchange. Species status for the populations has no reasonable alternative.

Since codominance is the rule for allelic variation at loci encoding proteins (it was observed for all of the protein variation within these samples), electrophoretic data can also be used to determine whether reproductive isolation is complete and

whether occasional mismating leads to introgression. Thus the absence from the San José de Ocoa samples of a single individual heterozygous for one or more of the six clear allelic differences suggests that introgression between the two species must be rare, if it occurs at all. The samples are large enough to show that F_1 hybrid individuals must be uncommon but not so large as to exclude their occurrence. Of course, failure to detect hybrid individuals does not eliminate the possibility of attempted hybridization, whatever its frequency, if the issue of such unions is inviable.

A single individual in sample 1 of *A. marcanoi* is the exception to complete divergence of the two species on the basis of 6-phosphogluconate dehydrogenase variants. A heterozygote for the common variant of both species, it is not an F_1 hybrid (no *A. cybotes* were collected at this locality). This situation cannot be explained, nor does it require explanation. In extensive comparisons of sibling species the characteristic protein variants of one are often found in low frequency in the other (e.g., Prakash, 1969; Ayala and Powell, 1972; Webster and Burns, 1973). Had larger samples and more populations been considered, there probably would be fewer loci indicating absolute separation.

Conspecific populations are quite similar, both throughout the small known distribution of *A. marcanoi* and between *A. cybotes* samples separated by 420 kilometers. The unsatisfactory indophenol oxidase results — some individuals in sample 1 have a variant like that of *A. cybotes* — provide the only evidence for significant differentiation within *A. marcanoi.* Samples 4a and 4b of *A. cybotes* are essentially identical and are similar to sample 5 for all but one polypeptide (Table 1). If sample 5 is accepted as representing *A. cybotes* from the region of the type locality, then, of the two species around San José de Ocoa, that with the red dewlap has been correctly treated as the new species. The difference between intraspecific and interspecific levels of similarity is expressed as values of Nei's *I* in Table 2.

In nearly all interspecific comparisons involving at least 15 proteins, one or more has allowed an individual to be identified with complete or almost complete confidence. For instance, diagnostic proteins giving species assignment with 99% or greater certainty were found in each of several extensive comparisons of *Drosophila* sibling species (Ayala and Powell, 1972). In this comparison of *A. marcanoi* and *A. cybotes,* 10 proteins are diagnostic by the same criterion. Joint consideration of several, par-

ticularly the six having very distinct variants, should be sufficient to assign any individual to either *A. cybotes* or *A. marcanoi.* In fact, while the 1970 sample from San José de Ocoa was divided without error on the basis of dewlap color, for the 1974 sample it was necessary to use the electrophoretic results to correct some of the casual field identifications of juveniles and females. Three *A. cybotes* were misclassed as *A. marcanoi* and one *A. marcanoi* as *A. cybotes.*

Although unnecessary in the analysis of *A. marcanoi* and *A. cybotes,* the magnitude of a genetic similarity coefficient like Nei's *I* can be used arbitrarily to determine whether two allopatric populations merit species status. The proteins merely provide another class of phenotypic information to be used according to established taxonomic procedure, but the genetic interpretation is usually retained. A criterion for species recognition can be established in the context of several studies of populations at diverse taxonomic levels, as judged by morphology or observed reproductive compatibility. Similarity values for conspecific populations generally exceed 0.9, and exceptions are often associated with insular isolates or other distinctive evolutionary situations (see Selander and Johnson, 1973, for a review of such data). Infraspecific taxa showing some reproductive isolation differ at 10 to 25% of their loci, which is 10 to 15 times as much divergence as between local populations within those taxa (Ayala *et al.,* 1974). I feel that a similarity value of 0.7 or less indicates so much genic divergence that it is a fairly conservative criterion for species status. On this basis *Anolis marcanoi* certainly qualifies for recognition as a separate species: in comparisons with *A. cybotes,* $\bar{I}$ is 0.62.

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Table 1. Polypeptide Variation Within and Between Populations of *Anolis marcanoi* and *A. cybotes*.[1]

Polypeptide, Variants[2]		Sample 1	2	3a	3b	4a	4b	5
	N:	9	26	42	10	20	13	4
Albumin	a					1.00	1.00	1.00
	b	1.00	1.00	1.00	1.00			
Protein A	a	1.00	1.00	1.00	1.00			
	b					1.00	1.00	1.00
Phosphoglucose Isomerase	a					.22	.38	
	b	1.00	1.00	.99	1.00	.75	.62	.88
	c			.01				
	d					.02		.12
Lactate Dehydrogenase–1	a						.04	
	b					.92	.96	1.00
	c	1.00	1.00	1.00	1.00			
	d					.08		
Lactate Dehydrogenase–2	a							.12
	b	1.00	1.00	1.00	1.00	1.00	1.00	.88
Isocitrate Dehydrogenase–1	a					1.00	1.00	1.00
	b		.04					
	c	1.00	.77	.95	.85			
	d		.19	.05	.15			
Malate Dehydrogenase–1	a							.12
	b	1.00	1.00	1.00	1.00	1.00	1.00	.88
Malate Dehydrogenase–2	a		.12					
	b	1.00	.98	1.00	1.00	1.00	1.00	1.00
Alcohol Dehydrogenase	a					.12	.04	.12
	b	1.00	1.00	1.00	1.00			
	c					.88	.96	.88
Glutamic Oxaloacetic Transaminase–1	a	1.00	1.00	.99	1.00	1.00	1.00	1.00
	b			.01				
6-Phosphogluconate Dehydrogenase	a							.62
	b	.06				1.00	1.00	
	c							.38
	d	.94	.98	1.00	1.00			
	e		.02					
Phospho-glucomutase–1	a	1.00	1.00	1.00	1.00			
	b					1.00	.92	1.00
	c						.08	

Table 1 — Continued

Phospho-	a	.06		.07				
glucomutase-2	b	.83	.83	.83	.85	.92	.81	1.00
	c					.02	.19	
	d	.11	.17	.10	.15			
	e					.05		
Peptidase	a	.17						
	b	.83	1.00	1.00	1.00			
	c					1.00	1.00	1.00
Fumarase	a					.02	.12	
	b	1.00	1.00	.99	1.00	.98	.88	1.00
	c			.01				
Indophenol	a			1.00				
Oxidase	b					1.00		
Hemoglobin	a			1.00				
	b					1.00		
Plasma Protein-1	a			1.00				
	b					1.00		

[1]Proteins B and C, leucine aminopeptidase, isocitrate dehydrogenase-2, α-glycerophosphate dehydrogenase, and glutamic oxaloacetic transaminase-2 were invariant.

[2]Electrophoretic mobility determines order in lists of variants, with 'a' the most distant from the origin.

Table 2. Normalized identity of genes (*I*) as computed from 21 genes for all pairs of samples.

Sample Number	2	3a	3b	4a	4b	5
1	.996	.998	.997	.622	.611	.624
2		.998	.999	.618	.608	.623
3a			.999	.614	.604	.619
3b				.617	.606	.622
4a					.996	.958
4b						.951

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